Study of Effect of Sugar Mill Effluent on Fenugreek (*Trigonella foenum-graecum*) Varieties

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Abstract—*The present study has been carried out to assess the water quality parameters of sugar mill effluent and their effect on plant growth and biochemical constituents of Fenugreek (Trigonella foenum-graecum) varieties (Kasuri) and (Pusa Bold). The physico-chemical parameters included colour, odour, pH, temperature, electrical conductivity (EC), total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total hardness (TH), total alkalinity (TA) and chloride (Cl^-). In the pot culture experiment, fenugreek plants were grown up to 21 days, in the soil irrigated with different concentration of sugar mill effluent like viz. (control, 0.5%, 1.0%, 5.0% and 10.0%) to investigate their effect on plant growth parameters such as germination percentage, seedling vigour, plant length, fresh weight and dry weight and pigments such as chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid. The higher sugar mill effluent dilutions (above 5%) were found to affect initial growth parameters and decreased biochemical contents, but at lower dilutions (up to 5%) favoured initial plant growth of both the plant varieties.*

Keywords*—*Biochemical Contents, Fenugreek, Plant Growth, Sugar mill effluent.

I. INTRODUCTION

During the last 50 years, India faced a rapid industrial growth to come into the category of developed nations. With the onset of rapid industrialization various environment related issues are there du e to the degradation in quality of the various components of environment. Water Pollution is one of them but water pollution is concentrated within a few sub-sectors, mainly in the form of generation of toxic wastes and organic pollutants. Most of the Indian rivers and fresh water streams are seriously polluted by industrial wastes or effluents which come along waste waters of different industries such as fertilizer, pulp and paper, textile, sugar mills, tanneries, distilleries, etc. The sugar industry, apart from being an important contributor to the economy of numerous countries including India, is also a major source of organic and inorganic wastes in water bodies. India is the second largest producer of sugarcane after Brazil in the world with 550 sugar mills and 220 million tons cane per year and total sugar production 13.5 million tons per year (**Kaur *et al*., 2010)**. Sugar industry is basically seasonal in nature and operates only for 150–210 days in a year (November to May) **(Kolhe *et al*., 2009)**. A considerable amount of water is used in sugar industry and subsequently a large amount of effluent of medium pollution range is discharged. Sugar industry effluent is reported to have several organic and inorganic contents in different concentrations. Sugar mills discharge large amount of wastewater having low pH with high concentration of suspended solids, dissolved solids, BOD and COD **(Sajani & Muthukkarupan, 2011)**. There are more than 20 types of industries including sugar industry falls under red category because of their potentiality in polluting the environment (**CPCB, 2016)**. The sugar industry generates about 7.5 million ton of molasses, 45 million ton of bagasse, 5 million ton of press mud and 40 million m^3 of spent wash **(Kumar 2003; Uppal, 2004)**. In our country a huge amount of waste water generated from sugar industries is discharged on land or into the water bodies. They are hazardous to aquatic plants, animals and human beings. However, some effluents at certain dilution are found to be beneficial for irrigation purposes **(Taghavi and Vora, 1994)**. Plant responses to sugar mill effluent has been studied and reviewed extensively by **(Vaithinathan *et al*., (2014), Elayaraj (2014)**.

Photo.1: Fenugreek seeds

From ancient periods spices are used to flavor and improve the taste of food recipes. Beside this they are used in cosmetics and medicinal preparations of Indian

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systems such as ayurveda and unani. Fenugreek/Methi (family: (Leguminosae); genus (Trigonella); species (Trigonella foenum-graecum L.) is widely used as green leafy vegetable **(Reddy and Borse, 2001)**. The leaves and young pods are used as vegetables and the seeds as condiments. It has also some medicinal value. Fenugreek seed is actually popular for its fiber and a rich source of soluble dietary fiber content. It contains 50 % fiber (30 % soluble fiber and 20 % insoluble fiber) **(Nazma *et al*., 2011)**. It prevents constipation removes indigestion stimulates the spleen and is appetizing and diuretic. The leaves are quite rich in protein minerals and Vitamin C **(Reddy and Borse, 2001)**. Fenugreek is known to have anti-diabetic, antioxidant, antibacterial, anti-neoplastic, gastroprotective, hepatoprotective, hyper-cholesterolemic and hypoglycemic properties due to its rich composition of phytochemicals **(Kor *et al*., 2013, Olaiya and Soetan, 2014).** Fenugreek contains phenolic and flavonoid compounds which help to enhance its antioxidant capacity **(Dixit *et al*., 2005)**. It is well known for its gum, fibre, alkaloid, flavonoids, saponin and volatile contents **(Meghwal and Goswami, 2012)**.

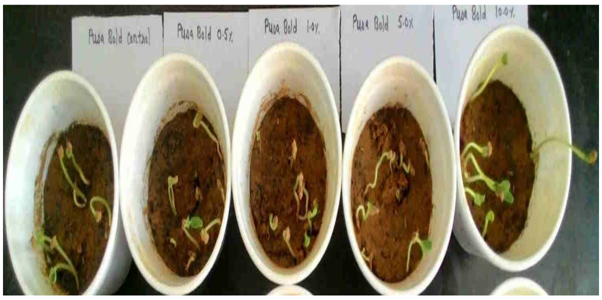

Photo.2: Fenugreek (Pusa Bold variety)

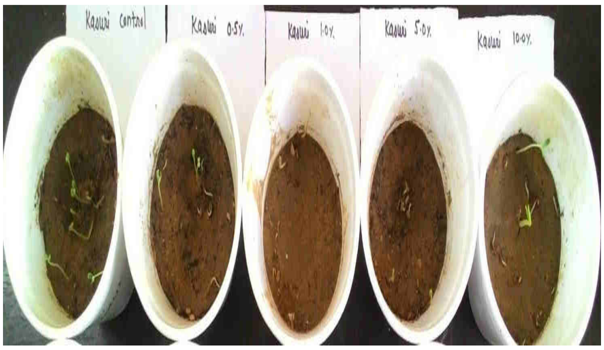

Photo.3: Fenugreek (Kasuri variety)

II. MATERIALS AND METHODS

Collection of sugar mill effluents

The effluent sample was collected inprecleaned, sterilized plastic containers from the outlet of the sugar mill situated at Bhali, Rohtak (Haryana co-op. sugar mills ltd) during May 2013 and were stored at 4^{o}C till further investigation as described by APHA (2010). Physico-chemical parameters, such as colour, odour, pH, temperature, electrical conductivity (EC), total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total hardness (TH), total alkalinity (TA) and chloride (Cl^-) were measured using standard methods **(APHA, 2010)**. Different concentrations i.e 0.0%, 0.5%, 1.0%, 5.0% and 10.0% were prepared by adding desired volume of canal water collected from Jui canal feeder, Bhiwani and used for the proposed pot trials.

Selection of seeds

Commercially available seeds of fenugreek (*Trigonellafoenum-graecum*) varieties (Kasuri) and (Pusa Bold) were procured from local market, Bhiwani, used in the study. Seeds with uniform size, colour and weight were chosen for the experimental purpose.

Pot culture experimental set up

The pot culture experiment was conducted in the laboratory of Department of Energy and Environmental Sciences, Chaudhary Devi Lal University, Sirsa. The effect of sugar mill effluent on the initial growth parameters and pigments of the fenugreek were studied using disposable pots (8.5 cm height and 7 cm width), filled with air dried soil taken into separate pots.The soil used in the experiment was sandy loam in nature. The methodology of **Aery (2010)** was adopted for surface sterilization of the test seeds of both varieties with 0.1% mercuric chloride ($HgCl_2$) for 30 seconds and vigorously rinsed with DDW three times to remove traces of $HgCl_2$. 10 seeds were sowed in each pot in duplicate of each variety in different dilutions i.e control, 0.5%, 1.0%, 5.0% and 10.0% to study the response of both varieties of fenugreek. All pots were watered at regular interval.

Initial Growth analysis

The test plant samples of both varieties were harvested 21st days after sowing. Three plants from each replicate of pot were analysed for various initial growth parameters such as germination percentage, seedling vigour, plant fresh weight, dry weight and plant length by following the methodology of **Aery (2010)**.

Germination percentage

The number of seeds germinated in each treatment was observed on 7th DAS (day after sowing). The total germination percentage was calculated by using the following formula:

Germination percentage =

$$\frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Seedling vigour index

Vigour index of the seedling were calculated by using the formula proposed by **Aery (2010)**.

Seedling vigour index =

Germination percentage × seedling length

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Pigment analysis

Leaves of the treated and control plants of both the test variety were used for the spectrophotometric estimation of chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid as per **Aery, 2010**.

Estimation of chlorophylls and carotenoids

The chlorophylls and carotenoid contents of primary leaves were estimated with 80% acetone with help of spectrophotometer (UV-1800, Shimadzu) **(Aery, 2010).**

$$\text{Chlorophyll 'a' (mg g}^{-1}) = \frac{12.7\,A_{663} - 2.69\,A_{645} \times v}{1000 \times w}$$

$$\text{Chlorophyll 'b' (mg g}^{-1}) = \frac{22.9\,A_{645} - 4.68\,A_{663} \times v}{1000 \times w}$$

$$\text{Total chlorophyll (mg g}^{-1}) = \frac{27.8\,A_{652} \times v}{1000 \times w}$$

Where v = volume of extract in ml, w = Fresh weight of leaf sample in gm

A_{663}, A_{645}, &A_{652} are the absorbance values at wavelengths of 663, 645 and 652 respectively.

$$\text{Carotenoids (mg g}^{-1}) = \frac{7.6\,D_{480} - 1.4\,D_{510} \times v}{1000 \times w}$$

Where v = final volume of extracting solution in ml, w = dry weight of tissue extracted in gm

D_{480} & D_{510} are the optical density at wavelengths of 480 and 510.

Statistical analysis

The data were subjected to mean, standard deviation and one way ANOVA (analysis of variance) using SPSS ver. 20 software.

III. RESULTS AND DISCUSSION

In the present study sugar mill waste water which was used for irrigation of fenugreek (*Trigonella foenum-graecum*) varieties (Kasuri) and (Pusa Bold) was analysed to know the physico-chemical parameters of waste water and its effect on initial growth parameters and biochemical parameters. The results of physico-chemical properties of sugar mill effluent along with the standard tolerance limits for industrial effluents discharged into inland surface water **(ISI, 1974)** are presented in **Table-1**.

The analysis of sugar mill effluent showed that it is acidic in nature with dark brown in colour. The acidic nature of sugar effluent might be due to the use of sulphuric acid and phosphoric acid during sugarcane juice clarification as discussed earlier by **(Memon *et al*., 2006, Ayyasamy *et al*., 2008)**. Dark brown colour could be due to presence of melanoidin which is the product of sugar amine condensation and decaying smell due to presence of indole and sulphure compounds **(Rath *et al*., 2010).** It contained fewer amounts of total suspended solids (180 mg/l) and higher amount of total dissolved solids (1920mg/l) meanwhile recommended values are 100mg/l and 2100mg/l respectively. The high value of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) indicate presence of high organic load which was 950 mg/l and 3125 mg/l respectively and the recommended values of BIS for BOD and COD are 30mg/l and 250mg/l. The presence of high amount of total hardness, alkalinity and chloride were also recorded **(Rathore *et al*. 2000, Borole and Patil, 2004)**.

Table.1: Physico-chemical analysis of sugar mill effluent and canal water

S.No	Parameters	Sugar Effluent	Canal water	ISI standards
1	Colour	Dark brown	Colourless	Colourless
2	Odour	Decaying smell	Odourless	Odourless
3	pH	4.2	7.63	5.5-9.0
4	Temperature	35°C	32°C	40
5	EC (µS)	2.858	0.18	-
6	TS (mg/l)	2100		2200
7	TDS (mg/l)	1920	120	2100
8	TSS (mg/l)	180		100
9	COD (mg/l)	3125		250
10	BOD (mg/l)	950	2.2	30
11	TH (mg/l)	190	60	300
12	Total alk. (mg/l)	340	110	-
13	Chloride (mg/l)	180	22	600

Growth estimation

The observation made on the effects of sugar mill effluent on growth parameters of Fenugreek (*Trigonella foenum-graecum*) varieties Kasuri and Pusa Bold are presented in **Table 2 & 3**. The results clearly indicate the growth parameters like germination percentage, seedling vigour, plant length, fresh and dry weight of both varieties varied with respect to different concentrations of sugar mill effluent.

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In Kasuri variety maximum value for germination percentage (95 ± 7.07) was observed in pot treated with 1.0% concentration of effluent where as in Pusa Bold variety maximum value for germination percentage (100 ± 0.0) was observed in 5.0% concentration as compared to control after 96 hrs. The results revealed that germination percentage of seeds decreased gradually as the concentration of effluent increased and at lower dilutions i.e 0.5% to 5.0% test plants responded in a better way in comparison of control plants showing the supply of essential nutrients needed for plant growth and metabolism.

Table.2: Growth parameters of fenugreek variety (Kasuri) under different concentration of the sugar mill effluent (Mean ±S.D.)

Treatment	***Germination %***	***Plant Length (cm)***	***Seedling vigour***	***Fresh weight (gm)***	***Dry weight (gm)***
Control	*80 ± 28.28*	*5.25 ± 0.35*	*425 ± 176.78*	*0.026 ± 0.009*	*0.002 ± 0.001*
0.5 %	*95 ± 7.07*	*6.3 ± 0.28*	*599.5 ± 71.42*	*0.021 ± 0.003*	*0.003 ± 0.002*
1.0 %	*90 ± 14.14*	*5.75 ± 0.21*	*516 ± 62.23*	*0.030 ± 0.004*	*0.002 ± 0.00*
5.0 %	*70 ± 14.14*	*5.15 ± 0.35*	*363 ± 97.58*	*0.023 ± 0.003*	*0.01 ± 0.001*
10 %	*70 ± 14.14*	*6.1 ± 0.28*	*551 ± 111.72*	*0.025 ± 0.003*	*0.001 ± 0.0*
F-Value	*0.897*	*5.643 **	*1.476*	*1.411*	*2.100*

** Significant at (α= 0.05)*

Table.3: Growth parameters of fenugreek variety (Pusa Bold) under different concentration of the sugar mill effluent (Mean ±S.D.)

Treatment	***Germination %***	***Plant Length (cm)***	***Seedling vigour***	***Fresh weight (gm)***	***Dry weight (gm)***
Control	*90 ± 0.0*	*9.55 ± 2.33*	*859.5 ± 210.01*	*0.076 ± 0.016*	*0.009 ± 0.004*
0.5%	*90 ± 14.14*	*10.50 ± 0.28*	*1127 ± 202.23*	*0.053 ± 0.007*	*0.009 ± 0.002*
1.0%	*95 ± 7.07*	*10.75 ± 1.20*	*1075 ± 120.21*	*0.077 ± 0.013*	*0.010 ± 0.003*
5.0%	*100 ± 0.0*	*12.50 ± 1.13*	*1183.5 ± 19.09*	*0.025 ± 0.006*	*0.010 ± 0.004*
10%	*80 ± 0.0*	*8.95 ± 1.77*	*1112 ± 33.94*	*0.124 ± 0.008*	*0.008 ± 0.002*
F-Value	*2.200**	*2.364*	*1.541*	*37.763 **	*0.399*

** Significant at (α= 0.05)*

The Pusa Bold variety resulted better in terms of early seed germination than Kasuri variety showing the inter-varietal difference. In Pusa Bold variety maximum values for seedling vigour (1183.5 ± 19.09), fresh weight (0.124 ± 0.008), dry weight (0.010 ± 0.003) and plant length (13.90 ± 0.42) were observed at 5.0%, 10.0%, 1.0% and 5.0% dilutions respectively whereas least values for seedling vigour (859.5 ± 210.01), fresh weight (0.025 ± 0.006), dry weight(0.008 ± 0.002) and plant length (9.55 ± 2.33) were observed in control plants, 5.0%, 10% dilution and control respectively.

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Germination percentage and fresh weight differ significantly (α= 0.05) in Pusa Bold variety.

In Kasuri variety maximum values for seedling vigour (599.5 ± 71.42), fresh weight (0.030 ± 0.004), dry weight (0.01 ± 0.001) and plant length (6.3 ± 0.28) were observed at 0.5%, 1.0% , 5.0% and 0.5% dilutions respectively whereas least values for seedling vigour (363 ± 97.58), fresh weight (0.021 ± 0.003), dry weight (0.001 ± 0.0) and plant length (5.15 ± 0.35) were observed at 5.0%, 0.5%, 10% and5.0% dilution respectively (Figure 2, 4, 5 & 6). The observation was conformity with **Medhi *et al*. (2008)**, **Vaithiyanathan *et al*. (2014)**. Plant length in Kasuri variety differ significantly (α= 0.05).

Pigment analysis

Chlorophyll estimation is one of the important plant parameters which are used as an index of production capacity of the plant. The chlorophyll content is an ecological index as well as growth parameters. Results of the effect of sugar mill effluent on biochemical contents of leaves of fenugreek plants were recorded on 21st day are presented in **Table 4 and 5.**

Table.4: Biochemical parameters of Kasuri variety under different concentration of the sugar mill effluent (Mean ±S.D.)

Treatment	Chl.a (mg g^{-1})	Chl. b (mg g^{-1})	Total chl. (mg g^{-1})	Carotenoid (mg g^{-1})
Control	0.102 ± 0.01	0.007 ± 0.001	0.079 ± 0.028	0.259 ± 0.136
0.5 %	0.115 ± 0.085	0.046 ± 0.002	0.082 ± 0.013	0.535 ± 0.198
1.0 %	0.137 ± 0.008	0.053 ± 0.021	0.147 ± 0.035	0.600 ± 0.028
5.0 %	0.095 ± 0.004	0.026 ± 0.008	0.107 ± 0.021	0.370 ± 0.028
10.0 %	0.067 ± 0.005	0.008 ± 0.001	0.040 ± 0.014	0.205 ± 0.021
F-Value	0.900	8.494*	5.447	4.894

** Significant at (α= 0.05)*

The photosynthetic pigments such as chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid content of crop plants decreased as the concentrations of sugar mill effluent increased. Maximum photosynthetic pigments such as chlorophyll-a (0.137 ± 0.008), chlorophyll-b (0.053 ± 0.021), total chlorophyll (0.147 ± 0.035) and carotenoid (0.600 ± 0.028) of Kasuri variety were observed at 1.0% concentrations of sugar mill effluent (Figure-6). Chlorophyll-b in Kasuri variety differ significantly (α= 0.05). In Pusa Bold variety maximum photosynthetic pigments such as chlorophyll-a (0.144 ± 0.087), chlorophyll-b (0.022 ± 0.018), total chlorophyll (0.153 ± 0.071) and carotenoid (0.440 ± 0.187) were observed at 5.0% concentration (Figure-7). When the data was subjected to ANOVA, It showed no significant difference with concentration in Pusa Bold variety.

Table.5: Biochemical parameters of Pusa Bold variety under different concentration of the sugar mill effluent (Mean ±S.D.)

Treatment	Chl. a (mg g^{-1})	Chl. b (mg g^{-1})	Total chl. (mg g^{-1})	Carotenoid (mg g^{-1})
Control	0.043 ± 0.013	0.010 ± 0.01	0.059 ± 0.008	0.231 ± 0.228
0.5 %	0.045 ± 0.020	0.012 ± 0.011	0.080 ± 0.023	0.273 ± 0.140
1.0 %	0.056 ± 0.023	0.017 ± 0.003	0.081 ± 0.0	0.350 ± 0.226
5.0 %	0.144 ± 0.087	0.022 ± 0.018	0.153 ± 0.071	0.440 ± 0.187
10.0 %	0.037 ± 0.007	0.010 ± 0.004	0.037 ± 0.009	0.268 ± 0.152
F-Value	2.273	0.578	3.281	0.645

** Significant at (α= 0.05)*

The increased chlorophyll content at lower concentrations (0.5% to 5.0%) may be due to sugar mill effluent at lower concentrations act as structural and catalytic components of proteins, enzymes and as cofactors for normal development of chlorophyll biosynthesis. At higher concentrations (10%), sugar mill effluent become toxic to plants and a decrease in the biochemical contents were observed. The observation was conformity with **Nagajyothi *et al*. 2008, Vijayaragavan *et al*. 2011**. Biochemical content decreased as the concentrations of sugar mill effluent increased creating oxidative effect in terms of production of reactive oxygen species such as superoxide radicals, singlet oxygen, hydrogen

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peroxides and hydroxyl radicals **(Sharma *et al*., 2012, Choudhary *et al*., 2013)** could cause cell death by lipid peroxidation, oxidation of proteins and inducing substantial damage to DNA **(Gill and Tuteja, 2010)**. Some of the possible reasons for the decrease in chlorophyll content may be the formation of enzyme such as chlorophyllase which is responsible for chlorophyll degradation **(Gupta *et al.* 2004; Saravanamoorthy and Ranjitha, 2005 and Nagajyothi *et al*. 2008)**. The increase in the carotenoid content in both varieties might be due to enhanced influence of nitrogen and other organic elements present in the sugar mill effluent **(Subramani *et al.* 1999)**.

Similar observations were also studied by many workers. **Hussain *et al*., 2013** conducted experiments on two maize cultivars and reported that when these crops were treated with different concentrations of sugar mill effluent, there was slight decrease in the growth at higher concentration but lower concentration (25%) of effluent was very effective in increasing the growth of both maize cultivars when compared with control. **Garg and Kaushik, 2006** reported that textile effluent did not show any inhibitory effect on seed germination of chickpea cultivars at a lower concentration. **Havaux, 2013** reported that reactive oxygen species, especially singlet oxygen, produced in the chloroplasts under stress conditions, can oxidize carotenoids leading to a variety of oxidized products, including aldehydes, ketones, endoperoxides and lactones. **Rani and Kumar, 2010** conducted work on *Triticum aestimum* and found promotory effect of different concentration of sugarcane industrial effluent on chlorophyll level, growth and yield of plant.

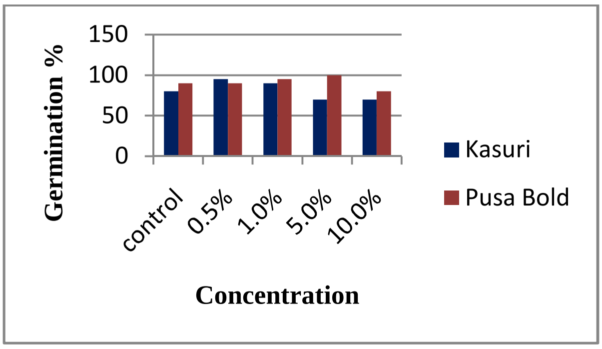

Fig.1: Impact of sugar mill effluent on germination (%)

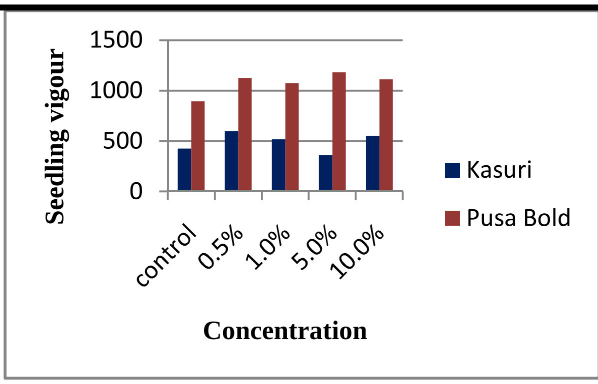

Fig.2: Impact of sugar mill effluent on seedling vigour

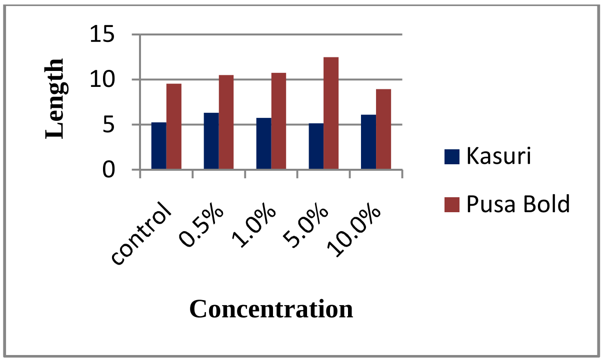

Fig.3: Impact of sugar mill effluent on plant length (cm)

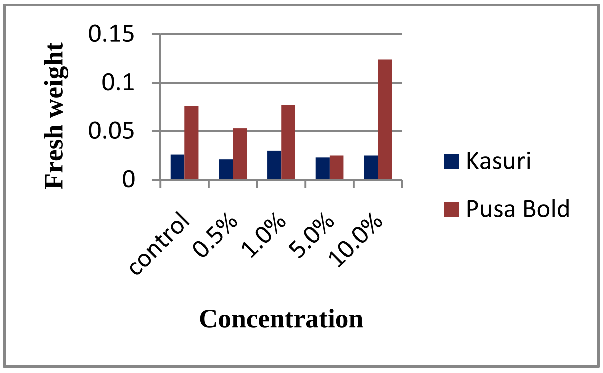

Fig.4: Impact of sugar mill effluent on plant fresh weight (gm)

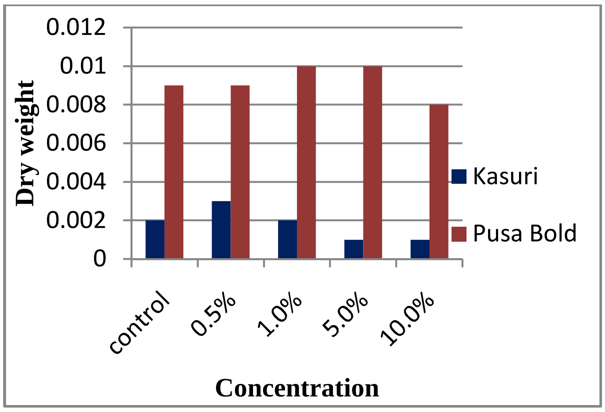

Fig.5: Impact of sugar mill effluent on plant dry weight (gm)

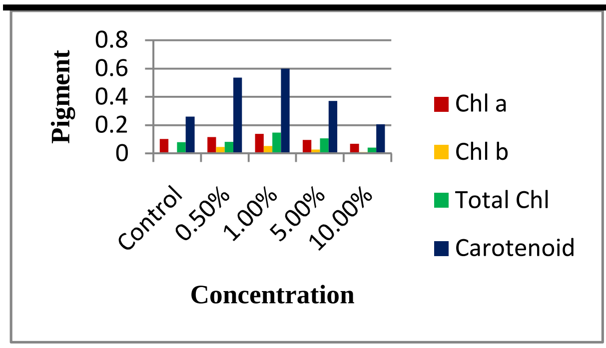

Fig.6: Impact of sugar mill effluent on pigment content of Kasuri variety

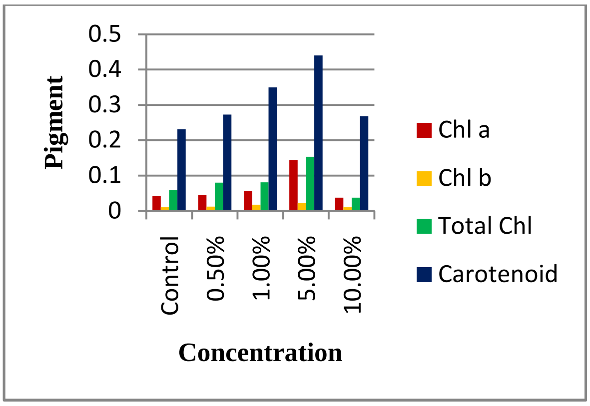

Fig.7: Impact of sugar mill effluent on biochemical content of Pusa Bold variety

IV. CONCLUSION

Among the effluent discharging industries, sugar mill plays a major role in producing a higher amount of water pollution because it contains large quantities of chemical elements. In the present study sugar mill waste water which was used for irrigation of fenugreek (*Trigonella foenum-graecum*) varieties (Kasuri) and (Pusa Bold) was studied to know the physico-chemical parameters of waste water and its effect on initial growth parameters and biochemical parameters.

From the conducted study, it is concluded that physico-chemical parameters such as COD, BOD, chloride, alkalinity, hardness, total dissolved solids were relatively higher in the sugar mill waste water and severly affected the Fenugreek (*Trigonella foenum-graecum*) plant growth. The initial plant growth parameters like germination percentage, seedling vigour, plant length, fresh and dry weight of both test varieties varied with respect to different concentrations of sugar mill effluent. The Pusa Bold variety resulted better in terms of early seed germination than Kasuri variety showing the inter-varietal difference. At lower dilutions i.e 0.5% to 5.0% test plant varieties responded in a better way in comparison of control plants showing the supply of essential nutrients needed for better cultivation, luxuriant and massive production of the plants of Fenugreek (*Trigonella foenum-graecum*). Observations recorded on the effect of sugar mill effluent on photosynthetic pigments such as chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid content of crop plants revealed that biochemical content decreased as the concentrations of sugar mill effluent increased due to oxidative effect. Where plant face oxidative effect in presence of industrial waste water and pigment system gets affected including carotenoid intensive biochemical and agronomic studies are needed to explore the adverse impact of sugar waste water on biochemical component responsible for medicinal property of Fenugreek. Diluted concentrations may be an adequate strategy for waste management by utilization of such type of Industrial effluent. Such type of low cost technology should be adopted for agricultural purpose.

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